THE EFFECT OF VARIOUS SUSPENSION MEDIA ON THE ACTIVITY OF CHOLINESTERASE FROM FLIES

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The cholinesterases (ChE's) of insects merit intensive study both because of their importance for normal nervous function in these animals and on account of their consequent role in the mode of action of certain highly effective insecticides (Roeder *et al.*, 1947; Dubois and Mangun, 1947; Chadwick and Hill, 1947; Roeder, 1948; etc.). Moreover, it is to be expected that the comparative study of enzymes of this type from diverse species will speed progress toward the definition and understanding of the significant properties of ChE's generally. We have therefore investigated the acetylcholine (ACh)-splitting mechanism in the nervous tissue of houseflies.

Several properties of this system have already been reported by Metcalf and March (1949, 1950) and by Babers and Pratt (1950, 1951). In their experiments with fly-head brei, these workers adopted suspension media commonly used with vertebrate ChE's and found such solutions compatible with the insect enzyme. Our assays were begun similarly, but it soon became apparent that maximal activity of fly-head ChE is not obtained under these conditions. It has seemed worthwhile for this reason to make a systematic study of the various factors which influence the rate at which ACh is hydrolyzed by fly-head brei. The present report is concerned mainly with the effects of variation in salt concentration. Some observations with solutions of glycerol and sucrose are included.

MATERIAL AND METHODS

1. *Material.* The data below refer to a strain of houseflies (*Musca domestica* L.) that was obtained early in 1948 from the testing stock of a commercial laboratory and that has been maintained in mass culture without exposure to insecticides or other toxicants. The larvae were grown at 32 degrees C. and approximately 30 per cent relative humidity (room conditions), in ground horsemeat placed between layers of sterile sawdust. The resulting pupae were blown free of sawdust and held in jars for emergence. Before mating had taken place, the adults were separated according to sex, and were kept in cages until use, with dry sugar, dry whole milk, and a source of water. Breeding stocks were housed in other cages and, in addition to the usual food and water, were supplied daily with ground horsemeat as an oviposition medium. The eggs or newly hatched larvae were then transferred to rearing jars. Under these conditions, development from egg to adult required 7 or 8 days.

2. *Preparation of tissues.* Adult flies of known age and sex were anesthetized with carbon dioxide gas. The heads were removed with fine scissors, counted into tared weighing bottles, weighed, ground, diluted to the desired concentration,

and assayed. Alternatively, batches of heads or whole flies were quick-frozen and stored indefinitely at -15 degrees C.; after thawing, they were processed at once. When necessary, tissue suspensions were held overnight or for longer periods in a refrigerator at 2 to 3 degrees C.

Breis were prepared from the heads by grinding them in a Pyrex test tube with 2 to 5 ml. of the desired suspension medium. A stainless steel pestle, rotated by a drill press at 700 r.p.m., provided a convenient and durable modification of the original all-glass Potter-Elvehjem homogenizer. Small samples, containing from 1 to 20 heads, were ground at room temperature for 30 to 35 seconds. Larger samples, up to 500 heads, were ground for longer periods, in a test tube surrounded by cracked ice. No abrasive was added.

3. *Method of assay.* Production of acid from ACh.Br was measured as proposed by Glick (1937), by means of titration with standard alkali at "constant" pH, using the Beckman Model G meter as a null instrument. The following conditions were adopted: tissue concentration, one head per ml.; final volume of sample, 20 ml.; reaction vessel, 50 ml. beaker; temperature, 25.0 degrees C.; pH, 8.0; rate of addition of 0.1 N NaOH during assay, 0.01 or 0.02 ml. as often as required to keep pH from dropping below 8.0; duration of measurement, approximately 15 minutes; beaker contents agitated by hand every 15 to 30 seconds; initial concentration of ACh.Br in assay mixture, 0.015 M.

Concentrated breis, containing 10 ground heads per ml., were made up in water, and two-ml. aliquots were diluted with stock reagent and water so as to provide a tissue concentration of one head per ml. in the desired strength of reagent. The reagent and sample containers were kept partially immersed on a shelf in the constant temperature bath. After addition of ACh.Br to the sample, pH was rapidly adjusted to slightly above 8.0 with 0.1 N NaOH or HCl. The pH was then allowed to fall to 8.0, at which time a stopwatch was started and 0.01 or 0.02 ml. of 0.1 N NaOH added by pipette. This raised pH to a value between 8.0 and (in extreme cases) 8.5; it was again allowed to fall, with stirring, and when 8.0 had been reached again the time was recorded to the nearest second and another 0.01 or 0.02 ml. of 0.1 N NaOH added. This routine was repeated as often as necessary for approximately 15 minutes. The rate of production of acid was then calculated from the elapsed time and the total volume of alkali used.

The reproducibility of the technique was measured by determining the activity of 20 aliquots, equivalent to 20 heads each, from a single sample of brei, which was suspended in a buffered solution of the following composition: NaCl, 26.30 gm.; KH_2PO_4, 3.85 gm.; NaOH, 1.00 gm.; H_2O, to one liter; approximate normality with respect to cations, 0.5; pH adjusted to 8.0. The average ChE activity of this preparation, after correction for acid produced from other sources, was 5.15 ± 0.23 micromoles ACh.Br hydrolyzed per head per hour. Thus, the coefficient of variability amounted to ± 4.5 per cent. Repetitions in unbuffered 0.5 N $MgCl_2$ and 0.5 N NaCl with 16 aliquots each gave a coefficient of ± 2.8 per cent in both cases. Variation was of the same order when 20-head aliquots were individually prepared. Such a test in buffer with 20 samples from a batch of male flies 5 to 6 days old yielded a coefficient of variability of ± 3.8 per cent.

4. *Reagents.* With the exception of glycerin, which was of USP quality, 98 per cent pure, reagents were of CP grade. Stock solutions were prepared with

de-ionized water.   The concentrations of the chlorides were checked by titration with 0.1 N AgNO₃.   Alkali employed for titrating acid that was produced by the samples was rechecked against standard 0.1 N HCl at intervals.   The loss of alkalinity amounted to less than 2 per cent in several months.   ACh.Br, recrystallized from the Paragon or Matheson product, was made up fresh daily at 0.15 M concentration in de-ionized water.

5. *Treatment of data.*   The rates reported represent enzymic hydrolysis, the

TABLE I

ChE activity of fly-head suspensions as a function of concentration of single salts, glycerol or sucrose

NaCl

Normality	0	0.0001	0.001	0.01	0.06	0.12	0.24	0.50	1.08	1.62	2.16	3.24
Activity in per cent*												
Average	100	105	107	122	188	196	222	242	238	213	190	121
Maximum	—	106	110	127	216	209	237	267	255	235	201	128
Minimum	—	104	105	118	177	174	200	200	221	192	182	114
Number of samples	7	5	5	5	5	5	5	5	5	5	5	5
Correction**	−0.56	−0.56	−0.56	−0.55	−0.52	−0.46	−0.50	−0.33	−0.26	−0.25	−0.24	−0.22

KCl

Normality	0	0.036	0.072	0.15	0.27	0.54	0.81	1.08	1.62
Activity in per cent*									
Average	100	168	192	218	242	248	247	231	204
Maximum	—	183	212	241	263	264	262	249	218
Minimum	—	150	171	162	209	221	230	217	170
Number of samples	7	5	5	5	5	5	5	5	7
Correction**	−0.56	−0.41	−0.41	−0.37	−0.33	−0.31	−0.27	−0.26	−0.24

MgCl₂

Normality	0	0.0001	0.001	0.01	0.058	0.115	0.23	0.46	0.92	1.84	3.68
Activity in per cent*											
Average	100	102	118	162	241	255	257	271	268	217	38
Maximum	—	106	121	164	255	268	279	296	277	235	47
Minimum	—	96	114	157	225	230	225	252	257	194	30
Number of samples	5	5	5	5	5	5	5	5	5	5	5
Correction**	−0.56	−0.56	−0.55	−0.47	−0.29	−0.29	−0.32	−0.34	−0.46	−0.70	−1.19

CaCl₂

Normality	0	0.051	0.102	0.20	0.41	0.81	1.63	3.26
Activity in per cent*								
Average	100	187	197	229	242	199	101	nil
Maximum	—	228	241	250	267	219	128	−1
Minimum	—	198	207	190	212	190	112	−16
Number of samples	5	5	5	5	5	5	5	5
Correction**	−0.56	−0.39	−0.41	−0.42	−0.46	−0.54	−0.71	−1.22

NaNO₃

Normality	0	0.06	0.12	0.24	0.50	1.08	1.62	2.16	3.24
Activity in per cent*									
Average	100	173	194	200	207	190	170	148	113
Maximum	—	176	198	204	216	194	174	151	123
Minimum	—	163	181	194	200	179	164	143	104
Number of samples	4	4	5	5	5	5	5	5	5
Correction**	−0.56	−0.38	−0.36	−0.35	−0.32	−0.32	−0.29	−0.25	−0.24

Glycerol

Molarity	0	0.125	0.25	0.50	1.00	2.00	4.00
Average activity in per cent*	100	99	97	93	88	70	46
Number of samples	2	2	2	2	2	2	2
Correction**	−0.56	—	—	—	−0.51	−0.54	−0.50

(used −0.56 for all concentrations)

Sucrose

Molarity	0	0.03125	0.0625	0.125	0.25	0.45	0.90	1.35
Average activity in per cent*	100	104	102	97	91	89	73	59
Number of samples	2	2	2	2	2	2	2	2
Correction**	−0.56	—	—	—	—	−0.47	—	−0.64

(used −0.56 for all concentrations)

* Activity expressed in terms of that of the same breis in water = 100 per cent, as follows: for NaCl, 2.70 micromoles ACh·Br hydrolyzed per head per hr.; for KCl, 2.67; for MgCl₂, 2.23; for CaCl₂, 2.38; for NaNO₃, 2.45; for glycerol and sucrose, 2.44.   Tissue concentration, one head per ml.

** Corrections (for acid produced from sources other than enzymic hydrolysis of ACh·Br) are in microequivalents per ml. per hr., and were applied to the raw data before calculation of per cent activity.   All runs at 25.0 degrees C.; pH, 8.0.

total acid production having been corrected by subtraction of the fraction contributed by processes other than enzymic breakdown of substrate in the particular medium concerned. These correction values were determined by titration of 20-ml. aliquots that contained the usual concentrations of tissue and ACh.Br, but whose ChE activity had been destroyed by incubation of the stock brei overnight or longer with 1×10^{-5} M di-isopropyl fluorophosphate (DFP).

Net measurements of enzymic activity were converted into micromoles of ACh.Br hydrolyzed per mg. fresh weight *and* per head per hour. Our results are reported only on the latter basis. The head and body weight of flies varies with age, among other factors, and there are changes in proportion as well as amount of water and other constituents. Moreover, a large and likewise varying fraction of the total weight consists of cuticle, which is presumably free of ChE activity. In contrast with these variations, ChE activity per head remains relatively constant, at a somewhat different level in each sex, after the first day or two of adult life. On this account, it has been advantageous to make comparisons on a per-head basis rather than with reference to fresh or dry weight, or protein or nitrogen content. The average fresh weight of a head in most of our samples has been between 1.2 and 2.9 mg. Dry weight in different batches ranged from 0.33 to 0.65 mg., and from 22 to 36 per cent of the total.

Results

Table I shows ChE activities of fly-head suspensions as affected by various concentrations of single salts, glycerol or sucrose. Comparison has been simplified by expressing the average rates as percentages of the activity found for the same breis in the absence of added reagent. Such treatment of the data is considered proper because the degree of activation or depression observed with a given concentration of reagent, although somewhat variable from one suspension to another, was found to be independent of the absolute level of activity of the preparation. The outcome of a fresh comparison of the salts at nearly optimal concentrations, in which all runs were made with aliquots of the same stock brei, is shown in Table II. Not listed is a single test with 0.5 N $(NH_4)_2SO_4$, in which ChE activity was about twice that of the same suspension in water. This salt solution was too strongly buffered to permit satisfactory measurement of the necessary correction. Results of a few other experiments are cited in the discussion.

Table II

Comparison of ChE activity of fly-head suspensions in 0.5 N solutions of single salts

Salt	NaCl	KCl	MgCl$_2$	CaCl$_2$
Activity in per cent*				
Average	212	201	229	208
Range	204–217	194–212	213–254	197–228
Number of observations	5	5	5	5

* Activity expressed in terms of that in water (1.78 micromoles ACh·Br hydrolyzed per head per hour) = 100 per cent. All determinations made on aliquots of the same brei, at 25.0 degrees C., pH 8.0.

Discussion

1. *Non-enzymic hydrolysis.* Under our conditions of measurement, at pH 8.0 and 25.0 degrees C., 0.015 M ACh.Br in buffered solution or in the higher concentrations of monovalent salts produced 0.22 to 0.24 microequivalents of acid per ml. per hr., in either the presence or absence of the usual concentration (one head per ml.) of DFP-inhibited tissue. These results compare reasonably well with values approximated by interpolation from the data of Augustinsson (1948) for non-enzymic hydrolysis of ACh.Cl and ACh.Br in bicarbonate buffer.

As may be seen from Table I, we observed and have used for correction considerably higher values in many of our experiments.

The observations with the salt solutions fall into two distinct groups. With the monovalent compounds, that portion of the total acid production that was not inhibited by DFP decreased more or less exponentially as salt concentration was increased. With the chlorides of divalent metals, on the contrary, an initial drop in acid production at low salt concentrations was followed by an increase that was related linearly to the concentration of salt.

In both cases, apparently, the higher rates at very low concentrations were the product of a technical shortcoming; namely, that addition of alkali during titration raised the pH of the medium temporarily and thereby accelerated the hydrolysis of ACh.Br. This effect was naturally more marked the smaller the buffering capacity of the medium; it was therefore greater in the more dilute solutions, where addition of 0.01 ml. of 0.1 N NaOH to a 20-ml. sample raised pH from 8.0 to 8.5 or occasionally even higher. Lower correction values were obtained with these dilute solutions when alkali was added in 0.0025 ml. amounts, but these results, of course, were not applicable to our experimental conditions.

The increase in acid production observed when concentration of $MgCl_2$ or $CaCl_2$ was above 0.1 N probably reflects a true increase in the rate of hydrolysis of ACh.Br. At pH 8.0 such solutions are quite strongly buffered, so that changes in pH on addition of alkali during titration become very small and cannot be held responsible for the effects noted. In the absence of substrate, tissue in 4.0 N $MgCl_2$ produced about 0.1 microequivalent of acid per ml. per hr. Ten times as much acid was released when substrate was present and tissue omitted. The increased hydrolysis in strong solutions of $MgCl_2$ is evidently not dependent on the presence of tissue, and it may be mentioned in passing that DFP-inhibited enzyme is not reactivated at all by incubation with either monovalent or divalent salts at 4.0 N concentration, even after several days. Other tests with 0.015 M ACh.Br in 4.0 N $MgCl_2$, where the loss of ester over periods of one to three hours in the presence of DFP-inhibited enzyme was determined chemically by the method of Hestrin (1949), indicated a hydrolysis rate of about 1.1 micromoles ACh.Br per ml. per hr. at 25.0 degrees C. and pH 8.0. These results may be compared with the correction value of 1.19 shown in Table I for 3.68 N $MgCl_2$.

The saddle-backed curve of correction values observed with the divalent salts seems, then, to owe its shape to a combination of two factors: (1) an inadequacy of procedure that resulted in slightly higher average pH during titration in very weak solutions; and (2) an opposing tendency toward acceleration of hydrolysis of ACh.Br as the concentration of divalent ions was increased. The latter process seems to be related linearly to salt concentration. We have assumed that similar

factors are concerned in the results with $CaCl_2$, although our attempt to analyze the situation has been confined to solutions containing $MgCl_2$.

The correction values obtained with glycerol and sucrose at all concentrations tested differed little from those observed in plain water, except for some indications of a trend toward higher rates of acid production at the higher concentrations of these compounds. This trend was more conspicuous in a few tests where substrate concentration was 0.06 M instead of the usual 0.015 M.

In concluding this section of the discussion, we would like to emphasize that, in some contrast with non-enzymic hydrolysis, the rate of breakdown of ACh.Br
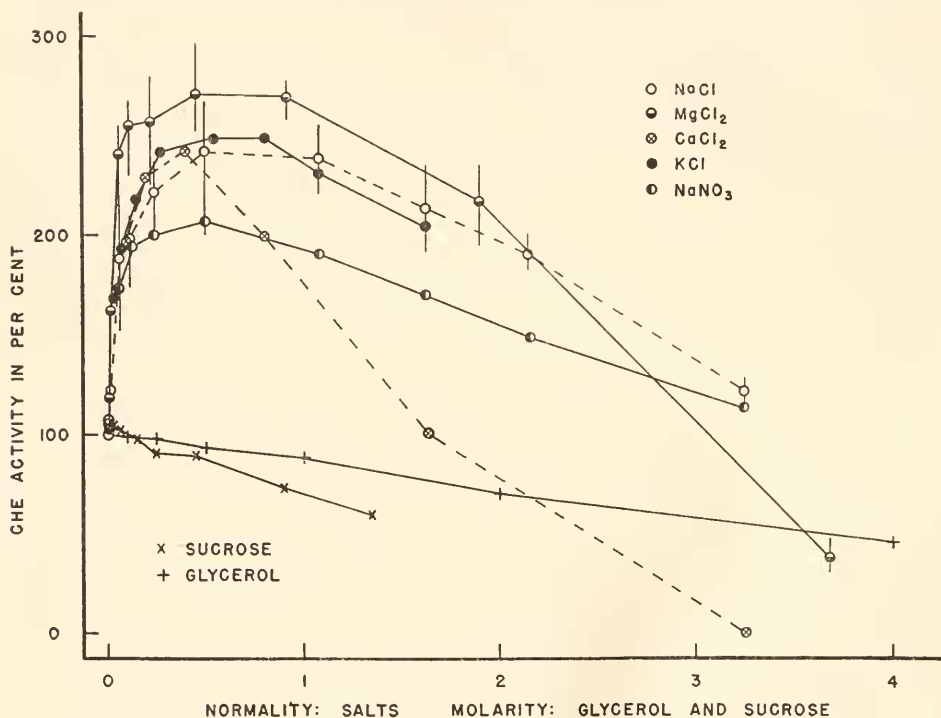

FIGURE 1. Relationship between ChE activity of fly heads and concentration of salt, glycerol or sucrose. Vertical bars indicate limits of variation encountered with NaCl and $MgCl_2$ solutions. For the range of variation with other salts, see Table I.

by ChE is but slightly affected by pH changes of as much as 0.5 unit in the neighborhood of pH 8.0, since activity at pH 8.5 is only 104 per cent of activity at 8.0. Use of correction values that were determined under conditions as nearly as possible identical with those prevailing during measurement of total activity therefore yields net results which should approximate closely the true rates of reaction between enzyme and substrate under the stated conditions. It is well to recognize that in certain circumstances the correction values may include acid production from a variety of sources other than what is usually thought of as non-enzymic hydrolysis, and that the rate of non-enzymic hydrolysis may be altered

several-fold as a result of details of technique or variation in the composition of the medium, even though pH and temperature are held within narrow limits.

2. *Variations in ChE activity.* From Tables I and II it is evident that added salt up to 0.5 to 1.0 N had a non-specific activating effect on fly-head ChE, and that this effect was reversed at still higher concentrations. With all the salts tested, the activity-concentration curves were quite similar when concentration was expressed in terms of normality, but the correspondence was less good when comparison was based on molarity or ionic strength. The maximal rate of enzymic hydrolysis was observed in all instances at salt concentrations ca. 0.5 N, and was some two to three times the value measured with water suspensions (Fig. 1).

Relationships of this type have not been reported for other ChE's, although certain scattered data are suggestive (*e.g.,* Alles and Hawes, 1940, with human erythrocytes). The somewhat discordant literature on the subject has been summarized by Augustinsson (1948), who ascribed the disagreements to the use of enzymes from varied sources. Undoubtedly, differences do exist in the response of various ChE's to the several ions, and for this reason it is perhaps surprising that Jandorf (1950), in studies of the esterase activity of chymotrypsin, obtained results strikingly similar to ours. However, in Jandorf's experiments activity seemed to be correlated with the ionic strength of the solutions.[1]

With the fly-head preparations, none of the ions tested appears to have the role of a necessary coenzyme. Aqueous suspensions, even as dilute as 0.1 head per ml., regularly yielded $Q_{ChE's}$ (at 25.0 degrees C.) of 15 to 25 mg. ACh split per 100 mg. fresh weight of tissue per hour. During dialysis against distilled or deionized water, loss of activity was slow, and not restored by added salts. Addition of any of the salts to these dialyzed preparations caused only the proportional change in activity already familiar from the studies with freshly prepared heads. Lack of specificity was likewise apparent in experiments with salt mixtures; for example:

Composition of medium:	water	0.5 N NaCl	0.5 N MgCl₂	0.25 N NaCl and 0.25 N MgCl₂
Activity in per cent:	100	258	275	263

In view of these various observations and the data in Tables I and II, it is unlikely that any ions present in the heads in a diffusible state (other than H^+ and OH^-) have an essential role in the activity of fly-head ChE. Nevertheless, slight differences in degree of activation were observed with some of the salts at equivalent concentrations. Thus, in both Tables I and II, as in the comparison cited in the preceding paragraph, it is apparent that the highest activity was found when $MgCl_2$ was present. The statistical significance of this difference was determined in an additional experiment. A stock brei was prepared, and 5 aliquots were run in water, 16 in 0.5 N NaCl, and another 16 in 0.5 N $MgCl_2$. The average (net) activity in water, taken as 100 per cent, was 2.32 micromoles ACh.Br hydrolyzed per head per hr. (range, 2.29 to 2.35). In 0.5 N $MgCl_2$, the

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[1] Results similar in some respects to ours have been reported recently by C. van der Meer. 1953. Effect of calcium chloride on choline esterase. *Nature,* **171**: 78–79.

average $\pm$ the standard error was 256 ± 1.8 per cent; and in 0.5 N NaCl, 232 ± 1.6 per cent. The difference is 24 ± 2.5 per cent, or more than 9 times its standard error. Thus, there is little room for doubt that $MgCl_2$ does have a slightly greater potency for activation than does NaCl, and it is likely that further investigation would disclose equally significant though small differences among some of the other salts. It is also clear from our data that, after the peak at about 0.5 N is reached, the ChE activity of suspensions containing $CaCl_2$ declines much more rapidly than it does in other salt solutions. Some specificity in the effects of the different cations has therefore been demonstrated, but this question will need re-investigation when purified enzyme is available.

The mechanism of the non-specific activation seen with all the six salts tested is not known. Enzyme solubility can hardly be concerned, since filtrates or supernatants from aqueous suspensions of ground heads were found to contain well over 90 per cent of the total activity and responded to addition of salts exactly as did the crude brei. Besides, salt concentrations above 1.0 N depressed activity without necessarily precipitating or destroying equivalent amounts of enzyme. Thus, for instance, when a suspension was made 5.5 N with NaCl and centrifuged, 86 per cent of the original activity was recovered in the supernatant. (Comparison was made in 0.5 N NaCl.)

The depression of activity seen with the higher concentrations of salts is, then, very largely reversible, as is the activation noted at lower concentrations. Possibly the reduction in reaction rate at high concentrations could be explained on the basis that the dense cloud of cations around the enzyme tends to block electronegative sites that are concerned in formation of an enzyme-substrate complex, in accordance with the theory advanced by Wilson and Bergmann (1950a, 1950b). But it is not easy to relate the progressive activation by salts in concentrations up to 0.5 N to this concept, although an indication that the observed changes in activity may involve some alteration of the enzyme itself is provided by the observation that a 4-fold increase in substrate concentration failed to accelerate the rate of reaction in 3.12 N NaCl. This fact argues against the interpretation that the reduced activity observed at this salt concentration was the result of direct interference with access of substrate.

The question may be raised, in view of the analysis by Wilson and Bergmann (1950a), whether changes in the ionic composition of the suspension medium may not cause shifts in the pS optimum and thus complicate activity comparisons of the sort we have made. Experiments undertaken to settle this point by determining the substrate optimum in three media, *viz.*, de-ionized water, 0.5 N NaCl and 3.0 N NaCl, gave somewhat inconsistent results on replication. In each instance, 5 runs were made at each substrate concentration in the series 0.1, 0.03, 0.01, 0.003 and 0.001 M. Despite the variation encountered, a trend toward increase in substrate optimum with increasing concentration of salt may be discerned in the averages (Table III) and we would estimate the respective optima provisionally as follows: water, 0.0016 M; 0.5 N NaCl, 0.0025 M; 3.0 N NaCl, 0.005 M. The corresponding enzymic activities, interpolated from the average data obtained at the concentrations actually tested, were in the ratio of 100:242:129. These are so close to the ratios observed with the same salt concentrations in Table I, where all runs were made with ACh.Br 0.015 M, as to leave no doubt that any

shift in pS_{opt}, with variation in salt concentration has played a negligible role in the comparisons with which this study is primarily concerned.

Glycerol was tested in order to provide a basis for future comparison of our observations with those of Babers and Pratt (1950), who used it in 30 per cent strength for preparation of stock breis. Since, in contrast to our experience with salts, we found that ChE activity was depressed progressively as concentration of glycerol was increased, we added a series of tests with sucrose, as another example of a non-electrolyte. Here, too, activity appeared to bear an inverse relationship to concentration, with the distinction that the slope of about -31 per cent per mole is appreciably steeper than the slope found with glycerol (about -14 per cent per mole).

TABLE III

Variation in activity of fly-head ChE in various media as a function of substrate concentration

Molar concentration of ACh·Br	0.001	0.003	0.01	0.03	0.10
	Average enzymic activity in micromoles per head per hour				
Suspension medium					
Water	2.46	2.32	1.89	1.99	2.12
0.5 N NaCl	4.82	4.91	4.40	3.51	2.49
3.0 N NaCl	2.96	3.22	2.99	2.86	2.37

Each series of observations is the mean of 5 replications.
All runs at 25.0 degrees C., pH 8.0.

Inasmuch as there seemed to be little likelihood that either of these compounds was acting as a chemical inhibitor, we were inclined to seek some physical mechanism, such as interference with diffusion of substrate toward or reaction products away from the active sites on the enzyme, as an explanation of the changes in activity observed. In such a case, the rate of hydrolysis might be expected, as a first approximation, to be related inversely to the viscosity of the medium. Unfortunately for this hypothesis, the viscosity of aqueous solutions of glycerol or sucrose does not vary linearly with concentration, whereas the depression of ChE activity did. Moreover, activity was depressed to about the same degree in 1.35 M sucrose, with a viscosity of about 5 centipoises, as in 4.0 M glycerol, whose viscosity is only some 2.5 centipoises. And in addition, as with the concentrated salt solutions, raising substrate concentration to 0.06 M did not yield greater activity in the presence of 4.0 M glycerol or 1.35 M sucrose; in fact, the rate of hydrolysis was reduced some 10 to 15 per cent in comparison with that obtained with 0.015 M ACh.Br, a result similar to what was seen with suspensions in plain water. Kodera (1928) also concluded that viscosity changes were not responsible for the inhibitory effect of gum arabic and soluble starch on the ChE's of human serum and red cells; and suggested that the test materials might have been adsorbed to the enzyme surface and thus have masked the active sites. His data, like ours with glycerol and sucrose, resemble the results with salt solutions to the extent that they indicate that the observed changes in activity are due to some alteration of

the enzyme. This could consist in blocking of the active sites as a consequence of adsorption or of some more fundamental alteration in the properties of the enzyme molecule.

Although further elucidation of the nature of these changes would be most desirable, it does not appear possible on the basis of present information. One may nevertheless draw the practical conclusion that, for experiments where maximal activity of fly-head ChE is desired, it will be well to have some salt present at about 0.5 N concentration, and to avoid the presence of compounds such as glycerol and sucrose.

The authors wish to acknowledge the kindness of Dr. Wm. E. Dove in supplying the pupae from which our fly culture was started.

Summary

1. The cholinesterase (ChE) activity of fly-head suspensions was measured titrimetrically at 25.0 degrees C. and pH 8.0 with acetylcholine bromide 0.015 M as substrate, as a function of the concentration of various single salts, glycerol, or sucrose. The species tested was *Musca domestica* L.

2. The salts, NaCl, KCl, $MgCl_2$, $CaCl_2$, $NaNO_3$, $(NH_4)_2SO_4$, at concentrations up to 0.5 to 1.0 N, had a non-specific activating effect. At still higher concentrations, activity was depressed progressively below the maximum, which was two to three times the value observed with water suspensions.

3. None of the ions tested was found essential to the activity of fly-head ChE. $MgCl_2$ was slightly but significantly more potent as an activator than NaCl.

4. Both glycerol and sucrose were depressant at all concentrations tested. The relationship was linear with concentration. For glycerol, the slope was about − 14 per cent per mole and for sucrose, about − 31 per cent per mole.

5. The depression of activity in the presence of higher concentrations of salts, glycerol or sucrose was not relieved by a four-fold increase in substrate concentration.

6. It is inferred that the changes in activity observed reflect alterations of an unknown nature in the properties of the enzyme, rather than direct interference with access of substrate to the active sites.

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